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Author(s): SHARON A. HALL , REBECCA L. ALLEN, RACHEL E. BAUMBER, LAURA A. BAXTER , KATE FISHER , PETER D. BITTNER-EDDY , LAURA E. ROSE , ERIC B. HOLUB AND JIM L. BEYNON

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Maintenance of genetic variation in plants and pathogens involves complex networks of gene-for-gene interactions.

Research Article

Sharon A. Hall^{*1}, Rebecca L. Allen^{*1}, Rachel E. Baumber*, Laura A. Baxter*, Kate Fisher*, Peter D. Bittner-Eddy*, Laura E. Rose^{**}, Eric B. Holub* and Jim L. Beynon^{*†}

*Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK.

**Section of Evolutionary Biology, University of Munich (LMU), Großhadernerstr. 2, 82152 Martinsried, Germany

†To whom correspondence should be addressed: Jim Beynon. Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK.

Tel: +44 (0)2476 574455

Fax: +44 (0)2476 574500

E-mail: Jim.Beynon@warwick.ac.uk

¹ Hall S.A. and Allen R.L contributed equally to this work.

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Abbreviations: RPP, Recognition of *H. arabidopsidis* (previously known as *Peronospora parasitica*.), ATR, *Arabidopsis thaliana* recognised, indel, insertion or deletion, UKID, identification number for *A. thaliana* accessions of the UK metapopulation.

SUMMARY

The *RPP13* resistance gene in *Arabidopsis thaliana* exhibits the highest reported level of sequence diversity among known *R*-genes. Consistent with a co-evolutionary model, the matching effector protein ATR13 (*A. thaliana*-recognised) from *Hyaloperonospora arabidopsidis* (previously known as *Peronospora parasitica*), reveals extreme levels of allelic diversity. We have isolated 23 new *RPP13* sequences from a UK metapopulation, giving a total of 47 when combined with our previous studies. We have used these in functional studies of the *A. thaliana* accessions for their resistance response to 16 isolates of *H. arabidopsidis*. We characterised the molecular basis of recognition by expression of the corresponding *ATR13* genes from these 16 isolates in these host accessions. This enabled us to determine which alleles of *RPP13* are responsible for pathogen recognition and whether recognition is dependent upon the *RPP13*/ATR13 combination. Linking our functional studies with phylogenetic analysis we determined that: 1) recognition of ATR13 is mediated by alleles in just a single *RPP13* clade; 2) *RPP13* alleles in other clades have evolved the ability to detect other pathogen ATR protein(s); and 3) at least one gene, unlinked to *RPP13* in *A. thaliana*, detects a different subgroup of *ATR13* alleles.

INTRODUCTION

A successful biotrophic pathogen must produce a range of pathogenicity effector proteins, which are targeted to the host cytoplasm to create a favourable environment for growth and reproduction. This may include suppression of the host immune system along with tailoring of host metabolism for parasite nutrition. In response, resistance (R) proteins in plants have evolved that detect the presence of the effector protein and initiate a defence response. As long as effector and R proteins provide a selective advantage to pathogen and host, respectively, they will be maintained.

Hyaloperonospora arabidopsidis (recently reclassified by Goker *et al.*, 2004) is an obligate biotrophic oomycete that causes downy mildew on *Arabidopsis thaliana*. Multiple R genes have been identified from *A. thaliana* that recognise specific isolates of *H. arabidopsidis* and several of these R genes have been cloned (Parker *et al.*, 1997; McDowell *et al.*, 1998; Botella *et al.*, 1998; Bittner-Eddy *et al.*, 2000; van der Biezen 2002; Sinapidou *et al.*, 2004). One of these R-genes, *RPP13*, encodes a member of the intracellularly-located R proteins, consisting of a coiled-coil domain, a nucleotide binding site and a leucine-rich repeat domain (CC:NBS:LRR). It is present as a highly diverse allelic series at a single locus and alleles of *RPP13* determine recognition of several *H. arabidopsidis* isolates (Bittner-Eddy *et al.*, 1999).

There are two proposed mechanisms of interaction between resistance proteins and pathogen effectors. In one, an R protein can interact directly with a pathogen gene product and trigger a resistance response. Such direct interactions involving R-proteins have been demonstrated in only a few cases (Scofield *et al.*, 1996; Tang *et al.*, 1996; Jia *et al.*, 2000; Dodds *et al.*, 2006). AvrPto from *Pseudomonas syringae* pv tomato, was shown to directly interact with the resistance gene product Pto (Scofield *et al.*, 1996; Tang *et al.*, 1996). However Pto is not a member of the LRR-containing class of R-proteins, but rather it encodes a

cytoplasmically-located protein kinase. The Avr-Pita protein from *Magnaportha grisea* and the Pita protein, a cytoplasmically-located NBS-LRR R protein from rice, have also been shown to interact directly in yeast and *in vitro* (Jia *et al.*, 2000). Avr-Pita is predicted to be a zinc metalloprotease and a mutation in the protease motif caused loss of resistance and failure to interact with the R protein, Pita. The flax rust avirulence protein AvrL567 has been shown to directly interact with the R gene product, L, from flax in a yeast two-hybrid system (Dodds *et al.*, 2006).

The second proposed mechanism, the guard model (van der Biezen and Jones 1998), posits that the resistance protein monitors the state of the target of a pathogen gene product and responds to changes in its state upon exposure to the pathogen. Thus the guard model implies that a direct interaction between an R-protein and a pathogen gene product is not required. This is exemplified in the interaction between the *A. thaliana* R protein RPM1 and the *A. thaliana* innate immune protein RIN4 (Kim *et al.*, 2005). In this example RPM1 acts as a guard to detect the phosphorylation of RIN4 by the *Pseudomonas syringae* effector protein AvrRPM1 (Axtell and Staskawicz, 2003; Mackey *et al.*, 2003).

The *RPP13* resistance gene in *Arabidopsis thaliana* exhibits the highest reported level of sequence diversity among known R-genes (Rose *et al.* 2004, Bakker *et al.*, 2006, Ding *et al.* 2007a) and we have shown that it is the LRR region that is under extreme levels of diversifying selection (Rose *et al.* 2004). A pathogen effector gene, *ATR13*, the product of which triggers RPP13-mediated resistance, also reveals extreme levels of allelic diversity (Allen *et al.*, 2004; Allen *et al.*, 2008). The high level of diversity observed in these two proteins may imply that there is a co-evolutionary battle between them and hints at direct protein-protein interaction. An alternative explanation is that the diversity observed is also driven by the interaction of RPP13 with effector proteins other than ATR13 and by the interaction of ATR13 with other R-proteins.

Our previous work (Allen *et al.*, 2008) with *ATR13* alleles revealed that recognition specificity for RPP13-Nd-1 resides in the C-terminal region of the ATR13 protein, but examination of 15 alleles of *ATR13* showed variation existing throughout the molecule. We hypothesised that this extended variation was due to interaction with other resistance genes not yet identified. In this current work, we have assessed the allelic diversity of *RPP13* and used a biolistic assay to determine whether the protein products of the allelic forms can recognise ATR13 protein variants. We show that: 1) only a single clade of *RPP13* alleles was responsible for recognition of ATR13, 2) an *RPP13* allele in a different clade recognised a novel ATR protein from *H. arabidopsidis* and 3) consistent with our hypothesis from our previous studies, other R-protein(s) recognised variants of ATR13. These data demonstrate that a simple gene-for-gene model cannot explain the allelic diversity seen at *RPP13* and *ATR13* and that host-parasite interactions can result in a network of genic interactions between co-evolving species.

RESULTS

Specific recognition of *ATR13* by *RPP13* is restricted to only a few *RPP13* alleles.

Previously, two alleles of *RPP13* were shown functionally to provide isolate-specific recognition of *H. arabidopsidis*: *RPP13-Nd-1* recognised isolates Maks9, Emco5, Aswa1 (Bittner-Eddy *et al.*, 2000), and Bico1 (Allen *et al.*, 2008) while *RPP13-Rld-2* recognised isolate Wela3 (Bittner-Eddy *et al.*, 2000). These *RPP13* alleles fall into distinct clades within the neighbour-joining tree (Fig 1). This suggests that if recognition capability of *ATR13* by *RPP13* is widespread among *A. thaliana* accessions, it must have arisen early on during the diversification of this gene and been conserved despite extensive protein evolution at this locus. Alternatively, if alleles such as *RPP13-Nd-1* and *RPP13-Rld-2* recognise different *ATR* proteins, then the sequence variation observed at *RPP13* may reflect convergent evolution operating at *RPP13*, for recognition of *H. arabidopsidis* isolates, involving distinct *ATR* proteins. To determine the capability of *A. thaliana* accessions to recognise alleles of *ATR13*, we selected a range of accessions from the UK metapopulation that represented the clades of the neighbour-joining tree. We tested these and the two accessions that contained previously characterised functional *RPP13* genes (*Nd-1* and *Rld-2*) for their recognition response to *ATR13* from 16 isolates of *H. arabidopsidis*, by transient expression in a biolistic assay. Fifteen of the 16 *ATR13* alleles encoded different protein variants (*ATR13-Emco5* and *ATR13-Goco1* were identical). Remarkably only five different recognition profiles were present among 35 *A. thaliana* accessions (Table 1), illustrated by Groups 1A, 1B, 2, 3 and 4.

Usually in the biolistic assay, recognition response is characterised by a complete macroscopic absence of the reporter gene product (Allen *et al.*, 2008). This archetypal *Nd-1*

profile (Group 1A) (maximum elicitation of cell death by five *ATR13* protein variants) was only found in one other accession, UKID34. We have previously shown that *RPP13-Nd-1* from the Group 1 cluster confers resistance to *H.arabidopsidis* isolates Aswa1, Emco5, Goco1, Maks9 (Bittner-Eddy *et al.*,2000). The *ATR13* gene from these isolates and from Bico1 was responsible for triggering resistance (Allen *et al.*,2004; Allen *et al.*,2008). Here we show that *RPP13*-UKID34 (Group 1A) is sequence identical to *RPP13-Nd-1* and a biolistic assay of accession UKID34, unsurprisingly, resulted in the same *ATR13* recognition profile (Table 1).

An intermediate response (Allen *et al.*,2008) is characterised by some appearance of the reporter gene product, but this is reduced by approximately one order of magnitude in comparison with the non-recognised response (Fig. 2). Four accessions (Group 1B) (UKID5, UKID36, UKID37 and UKID80) recognised the same *ATR13* protein variants as Group 1A, however recognition of *ATR13*-Maks9 was intermediate. Consistent with this, resistance to isolate Maks9 was also weak in cotyledons of these accessions, permitting low-level sporulation following inoculation with this isolate (data not shown).

We cloned *RPP13-UKID37* (Group 1B) and transformed the susceptible *A. thaliana* accession Col-5 with this gene. This transgenic line recognised the same *ATR13* alleles in the biolistic assay as *RPP13-Nd-1*, including the intermediate recognition of *ATR13-Maks9*, characteristic of Group 1B, demonstrating that *RPP13-UKID37* was responsible for this recognition. This transgenic line was inoculated with Bico1, Emco5 and Maks9 and a resistance phenotype was observed with all three isolates. This shows that like *RPP13-Nd-1* and *RPP13-Rld-2*, *RPP13-UKID37* is an allele that exists in the UK metapopulation capable of recognising isolates of *H.arabidopsidis*. Within the clade which contains *RPP13-Nd-1*, there are three alleles of *RPP13*, which encode three protein variants. *RPP13* from UKID36 and UKID80 are sequence identical to *RPP13-UKID37* and, by inference, are responsible for

recognition of ATR13. RPP13-UKID5 differs from RPP13-UKID36, RPP13-UKID37 and RPP13-UKID80 by a single amino acid and this polymorphism is shared with RPP13-Nd-1 and RPP13-UKID37. The UKID5 accession also shows the intermediate recognition of ATR13-Maks9, thus, it is likely that *RPP13-UKID5* is responsible for recognition of ATR13. Therefore, *RPP13* alleles of Group 1 accessions are able to recognise the same group of ATR13 proteins. The *RPP13* alleles of Group 1 accessions show 13 fixed nucleotide differences compared to the *RPP13* alleles from the other accessions lacking ATR13 recognition. Ten of these nucleotide differences encode amino acid changes and these are all localised to the LRR region of RPP13. Considering only Group 1 alleles, 36 nucleotide differences separate the alleles of Group 1A and Group 1B, of which 32 encode amino acid differences. However, these 32 amino acid differences are distributed throughout the protein posing a challenge for the rapid localisation of the amino acids variants that account for the phenotypic difference in Maks9 recognition between Group 1A and Group 1B alleles.

R-proteins other than RPP13 can recognise ATR13.

Group 2 accessions, UKID8 and UKID66, are resistant to isolate Hind2 and both accessions recognise ATR13-Hind2 in the biolistic assay. To determine if this recognition is conferred by alleles of *RPP13*, we crossed UKID8 with Nd-1 (which does not show a recognition response in the biolistic assay with *ATR13-Hind2*) and tested the F₂ progeny in the biolistic assay with *ATR13-Hind2*. Among 31 F₂ individuals, resulting from a cross between UKID8 and Nd-1, recognition of ATR13-Hind2 segregated 24 recognised and 7 unrecognised which is consistent with a 3:1 ratio ($\chi^2 = 0.10$, $p=0.75$) consistent with the presence of a single recognition gene or tightly linked genes. A molecular marker within *RPP13-UKID8* segregated independently (45% recombination) from ATR13-Hind2 recognition in the

biolistic assay, demonstrating that a resistance gene other than *RPP13* is responsible for recognition.

The Group 3 accessions, UKID44, UKID65 and UKID71, recognised four alleles of *ATR13* including *ATR13-Maks9*. To determine if this recognition is conferred by alleles of *RPP13*, we crossed UKID71 with Col-5 (which does not show a recognition response in the biolistic assay with *ATR13-Maks9*) and tested the F₂ progeny in the biolistic assay with *ATR13-Maks9*. Among 48 F₂ individuals, resulting from a cross between Col-5 and UKID71, recognition of *ATR13-Maks9* segregated 35 recognised and 13 unrecognised which is consistent with a 3:1 ratio ($\chi^2 = 0.11$, $p = 0.74$) consistent with the presence of a single recognition gene or tightly linked genes. The recognition phenotype of the F₂ population suggested a single resistance gene was responsible for the recognition of *ATR13-Maks9*. However, molecular markers for *RPP13-UKID71* segregated independently (58% recombination) from *ATR13* recognition implying that it is a resistance gene other than *RPP13* that is responsible for this recognition phenotype. In similar experiments, *RPP13-UKID44* did not co-segregate with *ATR13* recognition. Therefore, *A. thaliana* accessions UKID44 and UKID71 harbour resistance genes, other than *RPP13*, that recognise and trigger a resistance response to alleles of *ATR13*. This demonstrates that *ATR13-Maks9* is recognised both by these novel genes in Group 3 accessions and by alleles of *RPP13* in the Group 1 accessions.

Preliminary mapping data indicates that the novel resistance genes in UKID44 and UKID71 map to the same linkage group on chromosome 1. An interesting observation is that both UKID44 and UKID71 are susceptible to infection by the *H. arabidopsidis* isolate Maks9, which suggests that the recognition of the *ATR13-Maks9* allele, as observed in the biolistic assay, does not occur during infection by the pathogen. In addition it would appear that this novel resistance gene is capable of recognising *ATR13-Wela3*, which is not recognised by

the Group 1 accessions. ATR13-Wela3 is recognised by the same UKID71 x Col-5 F₂ individuals that recognise ATR13-Maks9. In this case the recognition observed in the biolistic assay is mirrored by the pathology, since UKID44 and UKID71 are both resistant to the Wela3 isolate.

***RPP13* is capable of recognising pathogen genes other than *ATR13*.**

The largest group (Group 4) contained 24 members of the UK metapopulation and Rld-2. These accessions did not recognise any *ATR13* allele so far tested in the biolistic assay. However, Rld-2 can recognise the pathogen isolate Wela3 (Bittner-Eddy *et al.*, 2000). The transgenic line HRI3860 :: *RPP13-Rld-2* (Bittner-Eddy *et al.*, 2000) (HRI3860 is an *A. thaliana* line susceptible to isolate Wela3) does not show recognition of ATR13-Wela3 in a biolistic assay, but does trigger a hypersensitive reaction in response to infection by isolate Wela3. *ATR13-Wela3* encodes a protein which is recognised by UKID44, UKID65 and UKID71, demonstrating that this allele is functional in the bombardment assay and its non-recognition phenotype is not due to a lack of protein expression. Therefore, RPP13-Rld-2 recognises a pathogen effector other than ATR13, revealing that multiple independent recognition specificities have evolved at the *RPP13* locus involving more than one pathogen protein.

DISCUSSION

The *RPP13* gene is under high levels of selective pressure resulting in highly diverse alleles (Rose *et al.* 2004, Bakker *et al.*, 2006, Ding *et al.* 2007a). The RPP13 protein belongs to the coiled coil: nucleotide binding site: leucine rich repeat (CC:NBS:LRR) class of intracellularly-located plant resistance proteins. The CC:NBS regions encoded by *RPP13* alleles were shown to be under selection for amino acid conservation, whereas the LRR was under extreme levels of diversifying selection (Rose *et al.* 2004).

ATR13, the pathogen protein that can elicit RPP13-mediated resistance in the host, also shows high levels of allelic variation (Allen *et al.*, 2008). This extreme variability of host resistance protein and pathogen effector suggests that these two proteins are under diversifying selection, in which changes in the ATR protein are favoured to avoid detection by RPP13 or other R proteins, presumably without compromising its fitness benefit to the pathogen. Here we describe results demonstrating that ATR13 recognition by RPP13 is restricted to a single clade of *RPP13* alleles. We observed that the recognition profiles of ATR13 by Groups 2 and 3 is due to a novel resistance gene (or genes) at other loci in *A. thaliana*. In previous studies, we have pinpointed the recognition of ATR13 by RPP13 alleles to relatively few amino acid positions in ATR13, although our collection of pathogen isolates show amino acid variation throughout the ATR13 protein (Allen *et al.*, 2008). Therefore an interaction between ATR13 and novel resistance proteins from Groups 2 and 3 could explain variation outside of the regions identified as important for recognition by RPP13. In the case of accessions UKID44 and UKID71, we observe recognition of *ATR13-Maks9*, in the biolistic assay, but when infected with the *H. arabidopsidis* isolate Maks9, a resistance response is not triggered. One interpretation of these data is that the *H. arabidopsidis* isolate Maks9 contains a suppressor of recognition between ATR13 and a resistance protein. Evidence for suppression in RPP/ATR interactions has also been observed in the RPP13/ATR13 interaction

(Sohn *et al.*, 2007) and in the RPP1/ATR1 interaction (Rehmany *et al.*, 2005). The expression of a suppressor of recognition of ATR13 would permit the persistence of ATR13 in the pathogen population, even in the presence of the cognate plant resistance protein.

The *RPP13-Rld* allele is unable to recognise *ATR13* alleles and most likely detects an alternative effector protein in *H. arabidopsidis* isolates such as Wela3. The presence of alleles conferring recognition specificity to different effectors from the same pathogen has previously been demonstrated at the *RPM1* disease resistance locus of *A.thaliana* (Bisgrove *et al.*, 1994; Grant *et al.*, 1995) and at the *L* locus in flax (Dodds *et al.* 2004) and also at the *Pto* locus in tomato (Ronald *et al.*, 1992; Kim *et al.*, 2002). Dual recognition of different pathogens by a single resistance gene has been reported for the *Mi* locus in tomato (Vos *et al.*, 1998). The presence of different haplotypes conferring recognition specificity to different pathogen species has been reported at the *RPP8/HRT* locus in *A. thaliana* (Cooley *et al.*, 2000). Each of these previous examples are consistent with a model that recognition is not restricted to a single interacting pair of genes, but involves multiple gene interactions between host and pathogen. In this respect, it will be interesting to determine whether *RPP13* recognition capability extends to other pathogens.

Maintenance of variable proteins in a single RPP-ATR pair could be driven by direct, reciprocal co-evolution at these loci. This model has been heavily influenced by studies of disease resistance in crop plants, which have been intentionally bred for disease resistance to particular pathogens. However, in this study we use accessions from a wild plant population and show that variation in ATR13 is countered in the plant through the deployment of multiple resistance proteins. This is intriguing as it greatly increases the potential of the host resistance proteins to respond to multiple pathogen targets creating a more robust defence strategy, but refutes the idea that this is based on exclusive gene pair co-evolution.

Elucidating the molecular mechanisms of R protein recognition of pathogen effectors is a major goal in host pathogen interaction studies. The two models for R protein function, direct interaction with a pathogen product or to guard a host protein and respond to the pathogen proteins effect on this target, predict different evolutionary outcomes. The direct interaction model predicts the maintenance of diversity at the loci controlling these interactions in hosts and pathogens, whereas diversifying selection is not explicitly advantageous under the guard model. Under the guard model, resistance may be stable and R proteins may display rather limited protein diversity as observed at the *Rps2*, *Rps5* and *Rpm1* genes in *A. thaliana*. Considering the extensive allelic diversity present at *ATR13*, we would therefore predict a direct interaction of ATR13 with RPP13. However, the fact that alleles from only one clade of *RPP13* recognise ATR13 and no yeast two hybrid interactions can be demonstrated between ATR13 and RPP13 (Hall and Allen, unpublished) may suggest that this interaction functions via the guard model. This is in contrast to the interaction between the resistance genes *L5*, *6,7* and *AvrL567* in the flax rust system, where direct interaction between host and pathogen components is matched by high levels of allelic diversity (Dodds *et al.* 2006). Alternatively, recognition of ATR13 by RPP13 may have evolved recently, and the observed allelic diversity of *RPP13* may instead be a consequence of co-evolution with other avirulence proteins. Consistent with this, RPP13-Rld-2 is capable of recognising a pathogen protein other than ATR13, and such capabilities could be harboured by the large number of UK metapopulation members of Group 4 accessions. It will be interesting to determine whether other functional alleles of *RPP13* have recently increased in frequency in local populations, or at larger geographic scales in populations of *A. thaliana*.

In our study, we have identified new components of the *A. thaliana*/*H. arabidopsidis* recognition system, which clearly broadens the opportunities to investigate *RPP13* and *ATR13* interactions. We are currently mapping these new resistance and effector genes and it will be

interesting to examine the variation in these novel genes. This system also provides an ideal context to explore the debate over the origin of polymorphisms in *R*-genes and the maintenance of allelic diversity in natural populations (Ding *et al.* 2007b; Holub 2001; Holub 2008).

EXPERIMENTAL PROCEDURES

The *A. thaliana* UK metapopulation collection.

The *A. thaliana* accessions used in this study were collected by E. Holub (Holub 2008). Rld-2 is as described in (Holub *et al.*, 1994). The Col-5::*RPP13*-*Nd*-1 and HRI3860::pBaRld-2-WT (denoted as HRI3860::RPP13-Rld-2 in this work) transgenic lines were generated as described (Bittner-Eddy *et al.*, 2000). The Col-5::*RPP13*-*UKID37* transgenic line was generated in the same manner.

Sequencing of the RPP13 from *A. thaliana*.

RPP13 alleles were sequenced from a series of overlapping PCR products which were generated using primers designed to the Col-5 *RPP13* sequence (see Supplemental Materials and Methods for primer sequences).

Sequence data from this article have been deposited with the EMBL/Genbank data libraries under accession numbers FJ624087-FJ624109 inclusive.

***H. arabidopsidis* isolates.**

All *H. arabidopsidis* isolates used in this study were collected by E. Holub from naturally infected *A. thaliana* populations within the United Kingdom. The collection locations are detailed in Supplemental Table 1.

Cloning the ATR13 alleles.

Cloning of ATR13 alleles was carried out as described (Allen *et al.*, 2004; Allen *et al.*, 2008).

***RPP13* molecular marker analysis.**

PCR products were generated using primers *RPP13-5* and *RPP13-7* and sequenced using the same primers as above (see Supplemental Materials and Methods for primer sequences).

Phylogenetic analysis.

Multiple sequence alignments were generated using ClustalW (Thompson *et al.*, 1994) and adjusted manually in MacClade 4 (Maddison and Maddison, 2000). The Neighbour joining tree was computed by PAUP* 4.0b10 (Swofford 2003). The tree was rooted using the *RPP13* orthologue from *A. arenosa*.

Biolistic analysis.

Biolistic assays were carried out as described (Allen *et al.*, 2004). Assays were repeated several times and at least 4 replicate shots per construct per experiment were carried out. Leaves were incubated for 16h before staining for β -glucuronidase.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Table S1 Geographical locations of *A.thaliana* accessions.

Table 1. Recognition responses between *A. thaliana* accessions and ATR13 as measured by transient expression in a biolistic assay.

<i>A. thaliana</i> accession	ATR13- AtHnd1	ATR13- Aswa1	ATR13- Bico1	ATR13- Cala2	ATR13- Camd5	ATR13- Emco5	ATR13- Emoy2	ATR13- Emwa1	ATR13- Goco1	ATR13- Hiks1	ATR13- Him2	ATR13- Him4	ATR13- Maks9	ATR13- Noks1	ATR13- Waco5	ATR13- Wela3
Group 1A																
Nd-1	0	+	+	0	0	+	0	0	+	0	0	0	+	0	0	0
UKID54	0	+	+	0	0	+	0	0	+	0	0	0	+	0	0	0
Group 1B																
UKID5	0	+	+	0	0	+	0	0	+	0	0	0	+	0	0	0
UKID56	0	+	+	0	0	+	0	0	+	0	0	0	+	0	0	0
UKID80	0	+	+	0	0	+	0	0	+	0	0	0	+	0	0	0
UKID57	0	+	+	0	0	+	0	0	+	0	0	0	+	0	0	0
Group 2																
UKID8	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0
UKID66	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0
Group 3																
UKID44	0	0	+	0	0	0	+	0	0	0	0	0	+	0	0	+
UKID65	0	0	+	0	0	0	+	0	0	0	0	0	+	0	0	+
UKID71	0	0	+	0	0	0	+	0	0	0	0	0	+	0	0	+
Group 4																
Rld-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID72	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UKID79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

+ indicates a recognition response, 0 indicates no recognition response,

outlined red box indicates an intermediate response. Coloured boxes denote

recognition group

Fig 1. Neighbour-joining tree of RPP13 nucleotide sequences inferred using PAUP*4.0b10.

The HKY85 substitution model was assumed. This model allows for unequal base frequencies and a different rate for transitions versus transversions. Bootstrap proportions of 1000 bootstrap replicates > 50% are indicated on the branches. The recognition capabilities of the *RPP13* alleles are indicated by colours as follows; RPP13 recognises ATR13 (red); RPP13 confers resistance by non-ATR13 recognition (brown); non-RPP13 recognition of ATR13 (unknown R-gene) (green). Black denotes no recognition except for * which denotes accessions not tested. For Rld-2, RPP13 resistance was demonstrated by inoculation of HRI3860::*RPP13-Rld-2* with Wela3

Fig 2. Recognition responses of ATR13 alleles by *A. thaliana* lines. A selection of representative examples of leaves bombarded with ATR13 alleles and stained for GUS. Three distinct phenotypes were observed, no response (N) gives rise to 300-1000 blue-stained cells per leaf, full response (F) generates less than 10 blue-stained cells and an intermediate response (I) gives 40-150 blue stained cells per leaf.